



TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Toxicology Quality Assurance and Procedures Manual

8.8 THC-COOH Procedure

8.8 THC-COOH PROCEDURE

8.8.1 Purpose

To qualitatively and/or quantitatively identify 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) in submitted evidence using liquid-liquid extraction followed by instrumental analysis with gas chromatography/mass spectrometry (GC/MS).

8.8.2 Specimen Requirements

Samples for analysis will be presumptively positive for cannabinoids. Acceptable samples for this analysis include blood and urine. For additional samples see Alternative Matrices (section 6.6).

8.8.3 Apparatus and Equipment

Disposable 15 mL culture tubes and screw caps
Disposable 5 mL conical centrifuge tubes and screw caps
Disposable 10 mL culture tubes
Volumetric pipettes and disposable tips
Assorted volumetric glassware
Disposable transfer pipettes
Sample mixer
Centrifuge
Evaporation station
11 mm autosampler vials, inserts, and caps
11 mm crimper
GC/MS, ChemStation software, compatible computer, and printer

8.8.4 Reagents and Standards

Negative blood, urine, or other matrix as needed
(+/-)-11-nor-9-carboxy-delta-9-THC certified reference standard
(+/-)-11-nor-9-carboxy-delta-9-THC-D₃ certified reference standard (internal standard)
Water (H₂O)
Acetonitrile (C₃H₂N)
Ammonium Hydroxide (NH₄OH)
1.0 N Hydrochloric Acid (HCl)
Hexane/Ethyl Acetate (9:1) (C₆H₁₄)/(CH₃COOCH₂CH₃)
BSTFA + TMCS, 99:1 (Sylon BFT) (N,O-bis(trimethylsilyl)trifluoroacetamide + trimethylchlorosilane)



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8.8.5 Standard and Reagent Preparation

The following are examples of how to prepare the standards and reagents used in this procedure.

8.8.5.1 Standards

THC-COOH Stock Reference Standard Solution [1,000 ng/mL] (A & B)

Pipette 500 μ L of (+/-)-11-nor-9-carboxy-delta-9-THC [100 μ g/mL] certified reference standard and dilute to 50 mL with an appropriate solvent (e.g., reagent alcohol, methanol, etc.).

THC-COOH Stock Reference Standard Solution [10,000 ng/mL] (A)

Pipette 1000 μ L of (+/-)-11-nor-9-carboxy-delta-9-THC [100 μ g/mL] certified reference standard and dilute to 10 mL with an appropriate solvent (e.g., reagent alcohol, methanol, etc.).

THC-COOH-D₃ Reference Standard Solution [1,000 ng/mL] (Internal Standard)

Pipette 500 μ L of (+/-)-11-nor-9-carboxy-delta-9-THC-D₃ [100 μ g/mL] certified reference standard and dilute to 50 mL with an appropriate solvent (e.g., reagent alcohol, methanol, etc.).

THC-COOH Working Reference Calibrator/Control Standard Solutions

To make the working reference calibrator/control standard solutions, add the following amounts to a final volume of 1 mL with blood or urine.

CONCENTRATION	AMOUNT USED	STD SOLUTION
15 ng/mL	15 μ L	1000 ng/mL
25 ng/mL	25 μ L	1000 ng/mL
50 ng/mL	50 μ L	1000 ng/mL
100 ng/mL	100 μ L	1000 ng/mL
150 ng/mL	150 μ L	1000 ng/mL
250 ng/mL	25 μ L	10,000 ng/mL
500 ng/mL	50 μ L	10,000 ng/mL
1000 ng/mL	100 μ L	10,000 ng/mL

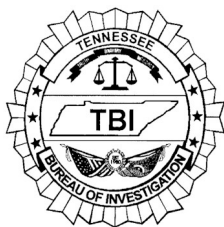
8.8.5.2 Prepared Reagents

1.0 N HCl

Add 82 mL concentrated hydrochloric acid to H₂O and dilute to 1000 mL.

Hexane/Ethyl Acetate (9:1)

Combine 90 mL hexane and 10 mL ethyl acetate.



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8.8.6 Procedure

1. Allow all reference standards and case samples to equilibrate to room temperature before beginning procedure.
2. Label, check, and load/unload all samples in accordance with the "Sample Pipetting Check List" (see Appendix section).
3. Prepare working reference calibrator and/or control standard solutions from stock reference standards. See example above.
4. Pipette 1 mL of corresponding case sample, calibrator, positive control, or negative control into the appropriately labeled 15 mL culture tube.
Note: Smaller sample volumes may be analyzed on a case-by-case basis.
5. Pipette 100 μ L of internal standard into each sample to make a final concentration of 100 ng/mL.
6. Add 2 mL of acetonitrile, mix approximately 60 seconds, and centrifuge until separated (approximately 5 minutes).
7. Decant supernatant into a clean, labeled 5 mL conical tube.
8. Add 100 μ L of NH_4OH , mix, and dry down sample in an evaporation station with heat and a dry gas (e.g. nitrogen) for approximately 20 minutes.
Note: Samples should be reduced to less than 1 mL, but not evaporated to dryness.
9. Add 2 mL of 1.0 N HCl.
10. Add 3 mL of hexane/ethyl acetate (9:1), mix/rotate sample for approximately 3 minutes, and centrifuge until separated (approximately 2 minutes).
11. Transfer the organic (upper) layer to a clean, labeled 10 mL culture tube and evaporate to dryness with heat (optional) and a dry gas (e.g. nitrogen) in an evaporation station. Discard bottom aqueous layer.
12. Reconstitute the residue with 35 μ L of BSTFA+TMCS, mix, transfer to an 11 mm autosampler vial with insert, and seal with cap.
13. Derivatize by heating for approximately 20 minutes.
14. Analyze and quantitate the samples by GC/MS (selected ion monitoring mode).

8.8.7 Reporting

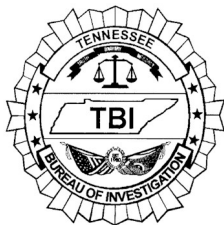
Results can be reported if the following criteria are met:

8.8.7.1 Ions 371, 374, 473, 476, 488 and 491 are present in the sample and used calibration standards and control(s).

8.8.7.2 Ion ratios for the sample must fall within $\pm 20\%$ of those of a calibrator or control standard of similar concentration.

8.8.7.3 The retention times of the 488 (THC-COOH) and 491 (THC-COOH- D_3) ions in the sample are within $\pm 1\%$ of those of a calibrator or control standard of similar concentration.

8.8.7.4 THC-COOH concentrations below 15 ng/mL (the lowest calibration point) shall be reported as "not detected".



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8.8.7.5 THC-COOH concentrations greater than the highest calibration point where the results are necessary for interpretation in the case shall be handled using the following methods:

- Reanalyze using smaller sample amounts (including dilutions).
- Reanalyze using higher standard concentrations.

8.8.7.6 THC-COOH concentrations greater than the highest calibration point where the results are not necessary for interpretation in the case may be reported as "greater than...." the highest calibration point.

8.8.7.7 Results shall be reported in ng/mL and truncated to the whole number.

8.8.7.8 When a definitive conclusion cannot be made, the reason shall be documented on the report (e.g., "insufficient sample for analysis", "sample unsuitable for analysis", "results are inconclusive due to sample condition", etc.).

8.8.8 References

Christine R. Goodall & Barbara J. Basteyns, "A Reliable Method for the Detection, Confirmation, and Quantitation of Cannabinoids in Blood". Journal of Analytical Toxicology 19 (1995); 419-426.